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Cytochrome P450 in Pharmacogenetics: An Update

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Cytochrome P450 in pharmacogenetics: an update

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Abstract

Interindividual variability in drug disposition is a major cause of lack of efficacy and adverse effects of drug therapies. The majority of hepatically cleared drugs are metabolized by cytochrome P450 (CYP) enzymes, mainly in families CYP1, CYP2, and CYP3. Genes encoding these enzymes are highly variable with allele distribution showing considerable differences between populations. Genetic variability of especially *CYP2C9*, *CYP2C19*, *CYP2D6*, and *CYP3A5* is known to have clear clinical impact on drugs that are metabolized by these enzymes. *CYP1A2*, *CYP2A6*, *CYP2B6*, *CYP2C8*, and *CYP3A4* all show variability that affects pharmacokinetics of drugs as well, but so far the evidence regarding their clinical implications is not as conclusive. In this review, we provide an up-to-date summary of the pharmacogenetics of the major human drug-metabolizing CYP enzymes, focusing on clinically significant examples.

Keywords

CYP2C9, CYP2C19, CYP2D6, Cytochrome P450, Drug metabolism, Pharmacogenetics, Pharmacokinetics

Nonstandard abbreviations

AhR aryl hydrocarbon receptor

AS activity score

CNV copy-number variant

CPIC Clinical Pharmacogenetics Implementation Consortium

CYP cytochrome P450

DPWG Dutch Pharmacogenetics Working Group

IM intermediate metabolizer

IWPC International Warfarin Pharmacogenetics Consortium

MAF minor allele frequency

NM normal metabolizer

PM poor metabolizer

PPI proton pump inhibitor

SNV single-nucleotide variant

SSRI selective serotonin reuptake inhibitor

TCA tricyclic antidepressant

UM ultrarapid metabolizer

VKORC1 vitamin K epoxide reductase complex subunit 1

1. Introduction

The cytochrome P450 (CYP) enzymes are a superfamily of hemoproteins, which have crucial functions in the metabolism of both endogenous and xenobiotic compounds. In humans, 57 putatively functional genes and 58 pseudogenes have been described (Nelson, Zeldin, Hoffman, Maltais, Wain, & Nebert, 2004). Most of the genes, which are divided into 18 families and 44 subfamilies based on their sequence similarity, have endogenous roles such as metabolism of bile acids, eicosanoids and steroids (Nebert & Russell, 2002). Only about a dozen CYP enzymes belonging to families CYP1, CYP2 and CYP3 are important for drug metabolism (**Table 1**). However, the majority of clinically used drugs are metabolized by CYP enzymes (Zanger, Turpeinen, Klein, & Schwab, 2008), and many drugs are predominantly metabolized by a single CYP enzyme at clinically relevant concentrations in humans. Thus, intrinsic and extrinsic factors that influence CYP enzyme activity can have important effects on pharmacokinetics of drugs. For some drug-metabolizing CYP enzymes, in particular CYP2C9, CYP2C19, and CYP2D6, genetic variability explains a large proportion of variability in enzyme activity, whereas for some other CYPs, such as CYP1A2, and CYP3A4, this proportion is much smaller (Zanger & Schwab, 2013). This review highlights the current knowledge on the pharmacogenetics of drug-metabolizing CYP enzymes, as well as its clinical relevance.

2. Genetic variability of CYP enzymes

The drug-metabolizing CYP enzymes are genetically highly variable, in part due to low evolutionary constraint as many of them lack essential endogenous function. The variability is characterized by common and rare single-nucleotide variants (SNVs), as well as copy-number variants (CNVs), defined as duplications or deletions. The variants of *CYP* genes are defined by star (*) allele nomenclature for which the Human Cytochrome P450 (CYP) Allele Nomenclature Database has served as a central repository for over 15 years. The database has recently been transitioned to be managed by the Pharmacogene Variation (PharmVar) Consortium available at www.PharmVar.org

(Gaedigk, Ingelman-Sundberg, Miller, Leeder, Whirl-Carrillo, Klein et al., 2017). The functional status of the variant alleles can range from no function to increased function, and thus phenotypes ranging from poor metabolizer (PM) to ultrarapid metabolizer (UM) can be inferred from the genotype (**Table 2**). In order to standardize the terminology used to describe allele functional status and phenotype, a consensus paper was published by Clinical Pharmacogenetics Implementation Consortium (CPIC) (Caudle, Dunnenberger, Freimuth, Peterson, Burlison, Whirl-Carrillo et al., 2017). Lastly, to facilitate the transformation of pharmacogenetic knowledge into clinical practice and to provide evidence-based guidelines on how available genetic test results should be used to optimize drug therapy, several consortia have been established. Both CPIC and the Dutch Pharmacogenetics Working Group (DPWG) provide genotype-based dosing guidelines based on gene-drug pairs, with some differences in guideline development methods and the number of guidelines produced so far (Bank, Caudle, Swen, Gammal, Whirl-Carrillo, Klein et al., 2017).

3. CYP1A subfamily

3.1 CYP1A2

CYP1A2 belongs to the CYP1 family and its gene is located on chromosome 15 along with *CYP1A1* and *CYP1B1*. Their expression is regulated by the aryl hydrocarbon receptor (AhR) pathway. CYP1A2 is abundantly expressed in the liver (Zhang, Wang, Gao, Wei, Tian, Zhao et al., 2016) and it is involved in the metabolism of about 10% of clinically used drugs that are metabolized by CYP enzymes (S. F. Zhou, Wang, Yang, & Liu, 2010). In contrast, CYP1A1 and CYP1B1 are mostly extrahepatic, and are of lesser importance for drug metabolism. CYP1A2 is involved in the metabolism of e.g., agomelatin, caffeine, clozapine, duloxetine, propranolol, tizanidine and zolmitriptan, as well as some endogenous compounds, such as melatonin and estradiol. CYP1A2 activity is strongly affected by environmental factors. AhR mediated induction by smoking or food

components can markedly increase CYP1A2 activity. In contrast, oral contraceptives, fluoroquinolones, and fluvoxamine inhibit CYP1A2 to a clinically relevant degree.

Several SNVs have been identified that affect CYP1A2 function, but they only partially explain the variability in enzyme activity (Gunes & Dahl, 2008). The most common variants are located in the 5' flanking region, including *CYP1A2*1F* (defining variant rs762551, g.-163C>A) which has a high frequency in many populations (Y. Zhou, Ingelman-Sundberg, & Lauschke, 2017). On the other hand, nonsynonymous coding variants (*CYP1A2*2* to **21*) are rare. Caffeine is frequently used to probe CYP1A2 activity, as caffeine N3-demethylation to paraxanthine is selectively catalyzed by CYP1A2 (Fuhr, Rost, Engelhardt, Sachs, Liermann, Belloc et al., 1996). *CYP1A2* and *AhR* loci have been identified in several genomewide association studies to be linked with habitual coffee consumption (Amin, Byrne, Johnson, Chenevix-Trench, Walter, Nolte et al., 2012; Coffee and Caffeine Genetics ConsortiumCornelisByrneEskoNallsGanna et al., 2015; Cornelis, Monda, Yu, Paynter, Azzato, Bennett et al., 2011). *CYP1A2*1F* has been associated in many studies with increased inducibility of CYP1A2 by e.g., smoking (Sachse, Brockmoller, Bauer, & Roots, 1999), but otherwise its functional consequence is less clear. In any case, genetic effects on CYP1A2 activity might be masked by environmental factors, in particular by smoking and oral contraceptive use (Matthaei, Tzvetkov, Strube, Sehrt, Sachse-Seeboth, Hjelmberg et al., 2016), and the conclusions thus can depend on the population studied. Furthermore, earlier studies including only single variants might have missed more complex haplotypes including several variants. Taken together, the current available evidence does not support *CYP1A2* genotype-based dosing for any drug.

4. CYP2A and CYP2B subfamilies

4.1 CYP2A6

CYP2A6 represents about 4% of total hepatic microsomal CYP enzyme content, and it is also expressed in some extrahepatic tissues, such as lungs and trachea (Di, Chow, Yang, & Zhou, 2009; Ding & Kaminsky, 2003; Zhang et al., 2016). In comparison to other CYP enzymes, CYP2A6 has a small active site (Yano, Hsu, Griffin, Stout, & Johnson, 2005). It has a major role in the metabolism of coumarin, letrozole, nicotine, and tegafur, and it contributes to the metabolism of other drugs, such as artemisin, efavirenz, halothane, pilocarpine, and valproic acid.

CYP2A6 gene is highly variable, with over 40 star alleles described to date, along with additional suballeles (<https://www.pharmvar.org/gene/CYP2A6>, accessed 5 February 2018). The spectrum of *CYP2A6* variant alleles include SNVs (e.g., *CYP2A6**2, *6), gene deletions (e.g., *4A-H), a hybrid gene with CYP2A7 (e.g., *3, *12A-C), and gene conversions (e.g., *1B1-17) (McDonagh, Wassenaar, David, Tyndale, Altman, Whirl-Carrillo et al., 2012). In principle, CYP2A6 phenotype, ranging from poor to ultrarapid metabolizer, can be inferred from *CYP2A6* genotype. However, the effects of CYP2A6 variant alleles are known to be substrate specific (Fukami, Nakajima, Higashi, Yamanaka, Sakai, McLeod et al., 2005), and due to the high number of different variants and complex genetic architecture, variant allele carriers may be misclassified as e.g., wild-type due to limitations in genotyping methods used (Hoffman, Nelson, & Keeney, 2001). In addition, the distribution of variant alleles varies greatly between populations (Y. Zhou et al., 2017). The decreased function allele *CYP2A6**9 is common in all major populations (minor allele frequency (MAF) ranging from 8% in Africans to 23% in East Asians), whereas *CYP2A6**17, *CYP2A6**23, *CYP2A6**25, and *CYP2A6**28 are only found in Africans, and *CYP2A6**7, and *CYP2A6**19 in East Asians. In Europeans, the most common variant alleles leading to decreased function are *CYP2A6**9, and *CYP2A6**35 with MAFs of 11% and 15%, respectively.

The metabolism of nicotine to cotinine is strongly related to CYP2A6 activity, and *CYP2A6* variants largely explain inter-individual variability in the metabolism of nicotine to cotinine (Bloom, Hinrichs, Wang, von Weymarn, Kharasch, Bierut et al., 2011; Hukkanen, Jacob, & Benowitz, 2005). *CYP2A6* variant alleles associated with reduced nicotine metabolism have been linked with lesser likelihood of becoming smoker, lower cigarette consumption, and better ability to quit than normal metabolizers in many studies (McDonagh et al., 2012; Ray, Tyndale, & Lerman, 2009).

CYP2A6 is involved in the bioactivation of tegafur to 5-fluorouracil, and genetic variation of *CYP2A6* has been associated with altered tegafur metabolism, and also clinical outcomes in some studies (Kaida, Inui, Suda, Nakamura, Watanabe, & Chida, 2008; Kong, Lim, Nam, Kook, Kim, Ryu et al., 2009; Park, Kong, Nam, Choi, Kim, Lee et al., 2011). These results suggest that bioactivation of tegafur is impaired in CYP2A6 poor metabolizers, but the associations with clinical endpoints have not been replicated in all studies, and they may be dependent on the cancer type and combination treatment given (Tanner & Tyndale, 2017). In addition, decreased function alleles of *CYP2A6* have been associated with increased plasma concentrations of another anticancer agent, letrozole (Desta, Kreutz, Nguyen, Li, Skaar, Kamdem et al., 2011; Tanii, Shitara, & Horie, 2011). It remains to be determined, however, whether the genotype has an impact on the efficacy and side effects of letrozole. In addition, *CYP2A6* variants have been reported to have an influence on the pharmacokinetics of efavirenz and valproic acid, even though other enzymes contribute the elimination of these drugs (Tanner & Tyndale, 2017).

4.2 CYP2B6

Similarly to CYP2A6, the expression of CYP2B6 is highly variable, and it represents only a small percentage of the total hepatic microsomal CYP content (H. Wang & Tompkins, 2008; Zhang et al.,

2016). *CYP2B6* and *CYP2A6* are located in close proximity in chromosome 19, and their expression levels are correlated (Al Koudsi & Tyndale, 2010). In addition to the liver, CYP2B6 is also expressed in the brain (Miksys & Tyndale, 2004). It is involved in the metabolism of e.g., bupropion, cyclophosphamide, efavirenz, methadone, and nevirapine (Turpeinen & Zanger, 2012; H. Wang & Tompkins, 2008).

Currently, 38 *CYP2B6* variants with designated star alleles are described (<https://www.pharmvar.org/gene/CYP2B6>, accessed 5 February 2018). The most widely studied allele is *CYP2B6**6, (c.516G>T, c.785A>G) resulting in reduced expression, which is found with a MAF of 3% in Europeans, 6% in Africans, 3% in East Asians, 16% in South Asians, and 3% in admixed Americans (Y. Zhou et al., 2017). The most common variant allele in all major populations is *CYP2B6**9 (c.516G>T), but its functional significance is less clear. Alleles with increased function have also been described (*CYP2B6**4 and *CYP2B6**22), with a MAF of <2% in most major populations, with the exception of *CYP2B6**22 with a MAF of about 4% in Africans.

The nonnucleoside reverse transcriptase inhibitor efavirenz is metabolized predominantly by CYP2B6. Several studies have shown an association between *CYP2B6* genotype, in particular the reduced function *CYP2B6**6 allele, and efavirenz related adverse effects including central nervous system toxicity and hepatotoxicity (Vo & Varghese Gupta, 2016). On the other hand, the effect on clinical outcomes has not been as widely replicated. Genotype-based dose reduction of efavirenz in CYP2B6 poor metabolizers has been performed in some studies (Gatanaga, Hayashida, Tsuchiya, Yoshino, Kuwahara, Tsukada et al., 2007). Interestingly, even though CYP2A6 is a minor metabolic pathway for efavirenz in general, *CYP2A6**9 has been associated with increased efavirenz plasma concentrations in CYP2B6 poor metabolizers (Haas, Kwara, Richardson, Baker, Papageorgiou, Acosta et al., 2014). To date, no genotype-based dosing guideline has been published, but efavirenz is a potential candidate for such a guideline with emerging evidence.

*CYP2B6*6* has been associated with reduced metabolism of bupropion, a pro-drug which is activated by CYP2B6. The effect of *CYP2B6*6* allele on the efficacy of bupropion on smoking cessation has been shown in some, but not all, studies (Chenoweth & Tyndale, 2017; Lee, Jepson, Hoffmann, Epstein, Hawk, Lerman et al., 2007; Zhu, Cox, Nollen, Faseru, Okuyemi, Ahluwalia et al., 2012)). Pharmacokinetics of several other CYP2B6 substrates, such as cyclophosphamide, methadone and nevirapine have been reported to be influenced by *CYP2B6* genotype, but evidence regarding them is not yet conclusive (Zanger & Klein, 2013).

5. CYP2C subfamily

The CYP2C subfamily consists of four highly homologous genes *CYP2C18-CYP2C19-CYP2C9-CYP2C8*, which are located in this order from centromere to telomere on chromosome 10q23.33. Even though CYP2C18 mRNA is highly expressed in the liver, it does not get efficiently translated into protein (Läpple, von Richter, Fromm, Richter, Thon, Wisser et al., 2003). The other CYP2C enzymes are expressed in the liver in the order CYP2C9 > CYP2C8 > CYP2C19, and, to a lesser extent, in the small intestine (Zhang et al., 2016). CYP2C enzyme activities can be markedly altered by environmental factors. A variety of xenobiotics such as rifampicin, and hyperforin can induce the transcriptional expression of CYP2C genes in hepatocytes and thereby increase the metabolism of CYP2C substrates. Several drug-activated nuclear receptors including CAR, PXR, VDR, and GR have been implicated in this induction (Chen & Goldstein, 2009). Many drugs can also act as reversible or time-dependent inhibitors of CYP2C enzymes, such as clopidogrel and gemfibrozil for CYP2C8, fluconazole and miconazole for CYP2C9, and fluvoxamine and omeprazole for CYP2C19.

5.1 CYP2C8

CYP2C8 has been established as one of the key drug-metabolizing CYP enzymes during the last 15 years (Backman, Filppula, Niemi, & Neuvonen, 2016). CYP2C8 metabolizes a variety of xenobiotics

and drugs, including amodiaquine, dasabuvir, imatinib, paclitaxel, loperamide, montelukast, pioglitazone, repaglinide, and rosiglitazone, for example.

There are tens of SNVs, short deletions and essential splice site variants that have been found in the *CYP2C8* gene. Fourteen *CYP2C8* alleles have a designated star allele (<https://www.pharmvar.org/gene/CYP2C8>, accessed 31 Jan 2018). The alleles *CYP2C8**2, *3 and *4 account for the majority of nonsynonymous variability of *CYP2C8* in humans (Y. Zhou et al., 2017). *CYP2C8**2 (c.805A>T, p.Ile269Phe; rs11572103) occurs with an allele frequency ranging from about 10% to 37% in individuals with a sub-Saharan African ancestry. However, this allele is rare or absent in most Asian, European and admixed American populations. The *CYP2C8**3 allele consists of two nonsynonymous variants (c.416G>A, p.Arg139Lys; rs11572080, and c.1196A>G, p.Lys399Arg; rs10509681) that are in complete or nearly complete linkage. *CYP2C8**3 is also in linkage disequilibrium with the *CYP2C9**2 allele (Yasar, Lundgren, Eliasson, Bennet, Wiman, de Faire et al., 2002). In individuals with a European ancestry, the allele frequency of *CYP2C8**3 approximates 11% (Y. Zhou et al., 2017). In South Asian and admixed American populations the frequency of the allele is about 4% and 7%, respectively, while in Sub-Saharan African populations the frequency is generally very low, albeit highly variable. Like *CYP2C8**3, the *CYP2C8**4 allele (c.792C.G, p.Ile264Met; rs1058930) is more common in European than in other populations. In most populations, its frequency is lower (by a factor of about two) than that of *CYP2C8**3.

The clinical significance of *CYP2C8* pharmacogenetics seems to be limited (Backman et al., 2016). Most *in vitro* studies have suggested that the *CYP2C8**2, *3 and *4 alleles result in moderately decreased *CYP2C8* function. However, also opposite findings have been reported regarding the *3 and *4 alleles, for example with respect to the metabolism of cerivastatin (Kaspera, Naraharisetti, Tamraz, Sahele, Cheesman, Kwok et al., 2010). Many clinical studies have suggested an increased *CYP2C8* activity for carriers of the *3 allele. Individuals with the *CYP2C8**1/*3 genotype have had an approximately 40-50% lower repaglinide AUC than individuals with the

*CYP2C8*1/*1* genotype (Backman et al., 2016). In addition, the AUCs of rosiglitazone or pioglitazone have been about 20-40% lower in *CYP2C8*3* carriers than in noncarriers, with an apparent gene-dose effect. In accordance with the pharmacokinetic findings, *CYP2C8*3* has been associated with reduced glycemic response to rosiglitazone treatment in two studies in patients with type 2 diabetes mellitus (Dawed, Donnelly, Tavendale, Carr, Leese, Palmer et al., 2016; Stage, Christensen, Feddersen, Beck-Nielsen, & Brøsen, 2013). Furthermore, in a recent study, *CYP2C8*3* carriers (n = 34) had a slightly higher imatinib metabolic ratio than did *CYP2C8*1/*1* (n = 147) patients (Barratt, Cox, Menelaou, Yeung, White, Hughes et al., 2017).

In contrast to the clinical findings with repaglinide and thiazolidinediones, the *CYP2C8*3* allele has been associated with a slightly impaired clearance of paclitaxel (Bergmann, Brasch-Andersen, Green, Mirza, Pedersen, Nielsen et al., 2011; Henningsson, Marsh, Loos, Karlsson, Garsa, Mross et al., 2005) and an increased risk for paclitaxel-induced neurotoxicity or myelosuppression (Backman et al., 2016; Boora, Kanwar, Kulkarni, Abyzov, Sloan, Ruddy et al., 2016; Lam, Frederiks, van der Straaten, Honkoop, Guchelaar, & Boven, 2016). Thus, it seems that the influence of *CYP2C8*3* on drug metabolism may be substrate dependent and is usually only modest. Based on limited evidence, *CYP2C8*2* and **4* alleles appear to have some, but limited, effect on the pharmacokinetics of CYP2C8 substrates. Inactive *CYP2C8* alleles, such as **5*, **7* and **11* are rare (Y. Zhou et al., 2017), but as case reports have shown, such alleles can drastically increase the risk of adverse reactions (Backman et al., 2016). At present, there are no genotype-based consensus guidelines for CYP2C8.

5.2 CYP2C9

CYP2C9 is one of the most important CYP enzymes in terms of the number of substrates; it has been estimated to contribute to the metabolism of approximately 15% of all drugs that are

metabolized by CYP enzymes (Daly, Rettie, Fowler, & Miners, 2017; Kirchheiner & Brockmöller, 2005). Important CYP2C9 substrates include warfarin (S-isomer), acenocoumarol, phenytoin, losartan, fluvastatin, bosentan, most sulphonylureas and several nonsteroidal anti-inflammatory agents.

Two nonsynonymous polymorphisms, rs1799853 (c.430C>T, p.Arg144Cys) and rs1057910 (c.1075A>C, p.Ile359Leu), define the *CYP2C9**2 and *CYP2C9**3 alleles, respectively (<https://www.pharmvar.org/gene/CYP2C9>, accessed 31 Jan 2018). They are the best studied alleles largely because they are functionally detrimental and globally the most common *CYP2C9* variants. Altogether, there are 60 *CYP2C9* variants with a star allele name and tens of other single nucleotide variants (SNVs) in the regulatory and coding regions of the *CYP2C9* gene. Apart from *CYP2C9**2 and *CYP2C9**3, some of the alleles are also relatively common and at least *CYP2C9**5, *CYP2C9**6, *CYP2C9**11, *CYP2C9**12 and *CYP2C9**13 can produce an enzyme with markedly reduced activity (Daly et al., 2017).

The allele frequencies of *CYP2C9**2 and *CYP2C9**3 are around 12% and 6% in European subjects (Y. Zhou et al., 2017). *CYP2C9**2 is also relatively common in admixed Americans (7%), South Asians (5%) and Africans (2%) but very rare in East Asians, while *CYP2C9**3 is remarkably common in South Asians (11%) and fairly common in East Asians and Americans (3-4%). *CYP2C9**3 is generally associated with a more than 80% reduction in CYP2C9 mediated intrinsic clearance, while the effect of *CYP2C9**2 is generally slightly smaller and varies considerably, depending on the substrate (Daly et al., 2017). Interestingly, *CYP2C9**8 and *CYP2C9**9 are more common (6% and 8%) than the *2 and *3 alleles in Africans, but extremely rare in Europeans (Y. Zhou et al., 2017). In addition, also *CYP2C9**5, *CYP2C9**6 and *CYP2C9**11 are much more common among Africans than among Europeans and *CYP2C9**14 is fairly common in South Asians, while all other alleles are rare in major populations. Based on genotypes, individuals can be classified as normal (*1/*1), intermediate (IM, carrier of one decreased-function allele) or poor (carrier of two decreased-function

alleles) metabolizers (**Table 2**). About 30% of Europeans are IMs and 4% are PMs, and the frequencies of these phenotypes are somewhat lower in other populations (**Table 3**).

One of the most sensitive drugs to genetic variability in CYP2C9 activity is warfarin, since its S-isomer is metabolized almost exclusively by CYP2C9 (Baker & Johnson, 2016; Rettie, Korzekwa, Kunze, Lawrence, Eddy, Aoyama et al., 1992). Consequently, the oral clearance of S-warfarin is reduced by 70-85% in CYP2C9 PMs and about 40% in IMs, leading to a longer S-warfarin half-life, longer delay in reaching a stable INR, lower warfarin dose requirement, and increased risk of bleeding during warfarin treatment, particularly in PMs (Jorgensen, FitzGerald, Oyee, Pirmohamed, & Williamson, 2012). Apart from *CYP2C9* variants (explain about 6% to 19% of variability in dose requirement), a number of other factors contribute to warfarin dose requirement, including vitamin K epoxide reductase complex subunit 1 (*VKORC1*) and *CYP4F2* genotypes, patient age, body size, smoking status and certain concomitant medications. Various algorithms have been developed to guide warfarin dosing on the basis of these factors. The first algorithm developed on a sufficient patient population was published 10 years ago by Gage et al. (Gage, Eby, Johnson, Deych, Rieder, Ridker et al., 2008) and is currently in use as a modified and updated web-based calculator (www.warfarindosing.org). A large worldwide project, International Warfarin Pharmacogenetics Consortium (IWPC), also developed a definitive warfarin dosing algorithm using clinical and genetic data on about 5000 patients treated with warfarin, including different ethnicities (International Warfarin Pharmacogenetics Consortium, Klein, Altman, Eriksson, Gage, Kimmel et al., 2009).

Three large randomized controlled trials, termed CoumaGen-II, EU-PACT and GIFT, comparing the above warfarin dosing algorithms to various conventional non-genetic approaches suggested that genotype-based dosing can be beneficial when warfarin treatment is initiated (Anderson, Horne, Stevens, Woller, Samuelson, Mansfield et al., 2012; Gage, Bass, Lin, Woller, Stevens, Al-Hammadi et al., 2017; Pirmohamed, Burnside, Eriksson, Jorgensen, Toh, Nicholson et al., 2013). However, in the fourth randomized study, COAG, the time within therapeutic range in the

first 4 weeks of warfarin treatment was not improved by genotype-based dosing, and the outcome was even worse in the genotyped African-Americans than in the controls (Kimmel, French, Kasner, Johnson, Anderson, Gage et al., 2013). The poor result of the COAG study may be partly explained by that *CYP2C9* genotyping included only the *2 and *3 alleles, but not the common African alleles. Accordingly, more comprehensive algorithms have been developed, and the warfarindosing.org website now contains both the Gage algorithm and the IWPC algorithm and enables adjustments for *CYP4F2*, *CYP2C9**5 and *6. The CPIC guideline for pharmacogenetics-guided warfarin dosing and its annotations (<https://cpicpgx.org/guidelines/guideline-for-warfarin-and-cyp2c9-and-vkorc1/>) provide detailed instructions on how to use pharmacogenetic algorithms for warfarin dosing (Johnson, Caudle, Gong, Whirl-Carrillo, Stein, Scott et al., 2017). Apart from warfarin, *CYP2C9* genotype influences to some degree also the pharmacokinetics of the other coumarins acenocoumarol and phenprocoumon.

In addition to anticoagulants, *CYP2C9* *2 and *3 alleles can markedly reduce the clearances of sulfonylureas, particularly tolbutamide and glipizide (Kirchheiner & Brockmöller, 2005). Clinical studies and case reports have suggested that *CYP2C9* variant allele carriers are more sensitive to the hypoglycaemic effect of sulfonylureas and may be at risk of hypoglycaemia (Daly et al., 2017). In addition, *CYP2C9* variant alleles markedly affect the pharmacokinetics of a number of nonsteroidal anti-inflammatory drugs, including celecoxib, ibuprofen and flurbiprofen. There are also several studies suggesting that *CYP2C9* variant carriers are at an increased risk of NSAID-associated gastrointestinal bleeding (Figueiras, Estany-Gestal, Aguirre, Ruiz, Vidal, Carvajal et al., 2016).

The antiepileptic drug phenytoin has a narrow therapeutic index and complex pharmacokinetic characteristics, including saturable metabolism and dependence on *CYP2C9* activity (Caudle, Rettie, Whirl-Carrillo, Smith, Mintzer, Lee et al., 2014). Both *CYP2C9**2 and *CYP2C9**3 are associated with impaired clearance of phenytoin. Consequently, it has been reported that phenytoin maintenance doses are reduced by about 30% in heterozygous carriers of these *CYP2C9*

alleles and by 30-50% in homozygous carriers, compared to noncarriers. Furthermore, case reports suggest that CYP2C9 PMs are at increased risk for dose-related phenytoin toxicities. Accordingly, the respective CPIC guideline recommends a 25-50% reduction of phenytoin starting maintenance dose in decreased function *CYP2C9* allele carriers, with subsequent maintenance doses adjusted based on therapeutic drug monitoring or response (Caudle et al., 2014).

5.3 CYP2C19

CYP2C19 participates in the metabolism of many clinically relevant drugs such as benzodiazepines, proton pump inhibitors (PPIs), selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs) and voriconazole, and it is important for the metabolic activation of the prodrug clopidogrel.

CYP2C19 is highly variable with 35 defined haplotypes with a designated star allele so far (<https://www.pharmvar.org/gene/CYP2C19>, accessed 14 Jan 2018). The most common no function allele is *CYP2C19**2 (c.681G>A; rs4244285), occurring with an allele frequency of 18% in Africans and Europeans, and over 30% in Asian populations (Y. Zhou et al., 2017). Other decreased or no function alleles (*3-*8) are typically rare, with the exception of *CYP2C19**3 (c.636G>A; rs4986893) in East Asians with a MAF of about 7%. On the other hand, the increased function *CYP2C19**17 allele (c.-806C>T; rs12248560) results in enhanced transcription probably due to the modulation of GATA4 binding (Mwinyi, Hofmann, Pedersen, Nekvindova, Cavaco, Mkrtchian et al., 2010). It is common in Europeans and Africans (MAF about 23%), less common in admixed Americans and South Asians (12-14%), and almost absent in East Asians. Thus, CYP2C19 phenotype ranges from PM to UM (**Table 3**).

The clinical significance of CYP2C19 pharmacogenetics is well established. For example, clopidogrel is a prodrug, which is activated in two sequential oxidative steps involving several CYP enzymes, including CYP2C19 (Kazui, Nishiya, Ishizuka, Hagihara, Farid, Okazaki et

al., 2010). Many studies have indicated that the bioactivation of clopidogrel is impaired in CYP2C19 poor metabolizers and that these PMs have lower levels of clopidogrel active metabolite than normal metabolizers (Scott, Sangkuhl, Stein, Hulot, Mega, Roden et al., 2013). Moreover, there is substantial evidence linking *CYP2C19* genotype with clinical outcomes, especially in patients with acute coronary syndrome who have undergone percutaneous coronary intervention (Mao, Jian, Changzhi, Dan, Suihua, Wenyi et al., 2013; Mega, Simon, Collet, Anderson, Antman, Bliden et al., 2010). More recently, a meta-analysis of clopidogrel-treated patients with stroke or transient ischemic attack suggested a similar level of risk for vascular events than that reported for patients with coronary artery disease (Y. Pan, Chen, Xu, Yi, Han, Yang et al., 2017), and the risk seems to be attenuated in stroke patients treated with clopidogrel monotherapy (Sun, Li, Li, Zhang, Zhu, Liu et al., 2015; Tornio, Flynn, Morant, Velten, Palmer, MacDonald et al., 2018). On the other hand, the increased function *CYP2C19*17* allele has been associated with increased risk of bleeding in some studies (Sibbing, Koch, Gebhard, Schuster, Braun, Stegherr et al., 2010). Based on the current available evidence, alternative antiplatelet therapy is recommended for CYP2C19 IMs and PMs in the CPIC guideline (Scott et al., 2013).

There is also strong evidence on the influence of *CYP2C19* genotype on TCAs and SSRIs that are metabolized by CYP2C19 (Hicks, Sangkuhl, Swen, Ellingrod, Muller, Shimoda et al., 2016). Tertiary amine TCAs, namely amitriptyline, clomipramine, doxepin, imipramine, and trimipramine, are demethylated by CYP2C19 to corresponding pharmacologically active secondary amines (e.g., amitriptyline to nortriptyline), and both the tertiary amines and secondary amines are metabolized by CYP2D6 to less active hydroxy-metabolites. Tertiary amines have a stronger serotonergic effect, whereas secondary amines have a more pronounced noradrenergic effect, and thus changes in CYP2C19 activity can have an impact on clinical response or adverse effects of tertiary amines (Gillman, 2007). Both CYP2C19 poor and ultrarapid/rapid metabolizers are at risk of suboptimal response to tertiary amines, and other TCAs not metabolized by CYP2C19 are

recommended for these individuals in the CPIC guideline (Hicks et al., 2016). Furthermore, the SSRIs citalopram, escitalopram, and sertraline are metabolized by CYP2C19, and especially for citalopram and escitalopram, CYP2C19 UMs are at risk of therapeutic failure and PMs at increased risk of side effects if normal recommended doses are used (Hicks, Bishop, Sangkuhl, Müller, Ji, Leckband et al., 2015; Jukic, Haslemo, Molden, & Ingelman-Sundberg, 2018).

Most PPIs are metabolized by CYP2C19 and their pharmacokinetics are affected by CYP2C19 phenotype (Furuta, Ohashi, Kamata, Takashima, Kosuge, Kawasaki et al., 1998). Both CYP2C19 IMs and PMs have higher PPI plasma concentrations compared to NMs, and consequently, the success rate of *H. Pylori* eradication therapy depends on *CYP2C19* genotype (Zhao, Wang, Yang, Wang, Shi, Xu et al., 2008). In contrast, CYP2C19 UMs are at risk of treatment failure, and consequently dose escalation is recommended for esomeprazole, lansoprazole, omeprazole and pantoprazole in *H. Pylori* eradication in the UM patients according to the DPWG guideline (Sven, Nijenhuis, de Boer, Grandia, Maitland-van der Zee, Mulder et al., 2011). Also the triazole antifungal voriconazole is metabolized by CYP2C19, and choosing an alternative antifungal agent not metabolized by CYP2C19 is recommended for both rapid/ultrarapid metabolizers and poor metabolizers due to risk of subtherapeutic concentrations and risk of adverse effects, respectively (Moriyama, Obeng, Barbarino, Penzak, Henning, Scott et al., 2016).

6. CYP2D6

Although the protein abundance of CYP2D6 in the liver is only about 5% of the total hepatic CYP amount (Y. Zhou et al., 2017), CYP2D6 contributes significantly to the metabolism of roughly 25% of all drugs in clinical use (He, Chen, Zhou, & Zhou, 2015). CYP2D6 substrates include TCAs, SSRIs (paroxetine, fluoxetine, fluvoxamine), other psychiatric drugs (venlafaxine, aripiprazole, risperidone), atomoxetine, opioids (codeine, tramadol, oxycodone), antiemetics (ondansetron,

tropisetron), primaquine, tamoxifen, and cardiovascular drugs (metoprolol, timolol, propafenone). CYP2D6 is inhibited strongly by various drugs, including fluoxetine, paroxetine, terbinafine and moclobemide. Moreover, although it has been thought that CYP2D6 is not inducible, accumulating data indicate that transcriptional regulation of CYP2D6 may account for part of CYP2D6 variability (X. Pan, Ning, & Jeong, 2017).

The *CYP2D6* gene is located on Chr22q13.1 in close proximity to the nonfunctional pseudogenes, *CYP2D7* and *CYP2D8*. There are more than 100 variants with a designated star allele in the highly polymorphic *CYP2D6* locus, including CNVs (<https://www.pharmvar.org/gene/CYP2D6>, accessed 31 Jan 2018). Overall, the different variations confer no, decreased, normal, or increased function, leading to huge variability in enzyme activity between individuals and populations, ranging from PMs (individuals with two nonfunctional alleles) to UMs (usually carriers of more than two functional *CYP2D6* alleles due to gene duplication) (**Table 3**). The *CYP2D6* diplotypes can be translated into predicted more definitive phenotypes using the activity score (AS) system (Gaedigk, Sangkuhl, Whirl-Carrillo, Klein, & Leeder, 2017; Gaedigk, Simon, Pearce, Bradford, Kennedy, & Leeder, 2008). To calculate the activity score, alleles are grouped according to their functionality, and a value is assigned to each allele to calculate the AS of each diplotype: no-function (0) alleles (*3, *4, *4xN, *5, *6, *7, *8, *11, *12, *36, *40, *42, *56), decreased-function (0.5) alleles (*9, *10, *17, *29, *41, *44, *49), normal-function (1) alleles (*2, *35, *43, *45), and increased-function (2) alleles (*1xN, *2xN). Accordingly, the CYP2D6 phenotype can thus be inferred as poor (AS 0), intermediate (0.5), normal (1-2) or ultrarapid (> 2) metabolizer. There is lack of consensus concerning whether patients with an AS of 1.0 should be assigned a normal or intermediate phenotype, and in some reports, they have been classified as normal-slow metabolizers, and individuals with an AS of 1.5-2 as normal-fast metabolizers (Gaedigk, Sangkuhl, et al., 2017; Gaedigk et al., 2008).

CYP2D6 allele frequencies vary substantially between populations. The *CYP2D6**2 allele, conferring normal *CYP2D6* function, and the variant defining *CYP2D6**4 (rs3892097), causing a splicing defect and an inactive *CYP2D6* gene product, are frequent in European, African, South Asian, and admixed American populations, with allele frequencies of 27-36% and 12-16%, respectively (Y. Zhou et al., 2017). On the other hand, the inactive alleles *CYP2D6**3 (4%) and *CYP2D6**6 (2%) are only found in individuals of European ancestry, while the decreased function allele *CYP2D6**10 is almost exclusively found in African, South Asian, and particularly in East Asian populations, where it is the most common *CYP2D6* allele (59%). *CYP2D6**14 is only found in East Asian populations (2%), while *CYP2D6**17 (20%), *CYP2D6**29 (9%), and *CYP2D6**43 (2%) are African haplotypes. The duplicated increased-function alleles *CYP2D6**1xN and *CYP2D6**2xN occur with frequencies of 1–2% in whites and Asians but are much more common in certain African populations, in which their frequency can be up to 29%, and can be enriched in some smaller populations, such as the Finns (7%) (Pietarinen, Tornio, & Niemi, 2016). The frequencies of deletions (*CYP2D6**5) vary from 1% to 7% (Gaedigk, Sangkuhl, et al., 2017).

Codeine is a weak opioid analgesic, whose opioid effect is dependent on morphine formed by *CYP2D6* (Crews, Gaedigk, Dunnenberger, Leeder, Klein, Caudle et al., 2014). Accordingly, the rate of bioactivation and analgesic efficacy of codeine are markedly decreased in *CYP2D6* PMs and increased in UMs, even though clinical evidence from pain studies regarding the effect of *CYP2D6* genotype is still lacking (Somogyi, Collier, & Barratt, 2015). There are several case reports of severe or life-threatening adverse effects following standard codeine doses in *CYP2D6* UMs. Consequently, the CPIC guideline recommends using alternative analgesics in PMs and UMs, and codeine is contraindicated by the medicines agencies in known *CYP2D6* UMs. In addition to codeine, also other opioids, including tramadol, hydrocodone and oxycodone, are O-demethylated by *CYP2D6*. As O-desmethyiltramadol is mainly responsible for the opioid effects of tramadol, similarly to codein, *CYP2D6* PMs and UMs can be susceptible to treatment failure when tramadol is used

(Orliaguet, Hamza, Couloigner, Denoyelle, Lorient, Broly et al., 2015). However, the effect of genetic variability in CYP2D6 on the analgesic efficacy and risk of toxicity with oxycodone and hydromorphone seems to be smaller due to the variable significance of parent drug and metabolites to the opioid effects.

All TCAs are metabolized to less active hydroxylated metabolites almost exclusively by CYP2D6 (Hicks et al., 2016). Therefore, there is huge *CYP2D6* genotype dependent variability in their metabolic clearance. Accordingly, clinical studies have shown particularly for amitriptyline and nortriptyline that standard doses of TCAs can fail to produce sufficient drug concentrations in the systemic circulation in UMs, while the concentrations of TCAs may reach supratherapeutic levels in PMs, resulting in risk of therapeutic failure or intolerable adverse effects, respectively. The CPIC guideline therefore recommends avoiding TCAs in CYP2D6 UMs and PMs.

Of the SSRIs, fluoxetine, and particularly fluvoxamine and paroxetine are metabolized by CYP2D6 (Hicks et al., 2015). CYP2D6 UMs have been shown to be at risk of low or undetectable plasma paroxetine concentrations, which may be associated with an increased likelihood of therapeutic failure. Therefore, it is advisable to select an alternative antidepressant in UMs. CYP2D6 PMs, on the other hand have greater drug exposure to fluvoxamine and paroxetine, compared to NMs, and therefore it is recommended that their doses should be reduced by 30-50% in PMs, if alternative antidepressants cannot be selected. Fluoxetine itself is pharmacologically active, and it is converted to a major active metabolite, R-norfluoxetine by CYP2D6. Therefore, CYP2D6 PMs have high fluoxetine levels and low norfluoxetine levels, and their total sum may not vary to a clinically significant degree between CYP2D6 genotypes.

The 5-hydroxytryptamine receptor antagonists ondansetron and tropisetron, that are used as antiemetics, are metabolized to inactive metabolites to a significant degree by CYP2D6 (Bell, Caudle, Whirl-Carrillo, Gordon, Hikino, Prows et al., 2017). Based on some pharmacokinetic data, the metabolism and clearance of tropisetron is highly dependent on CYP2D6 phenotype, while the effect

on ondansetron pharmacokinetics is only moderate. More importantly, there is clinical evidence showing that CYP2D6 UMs are at risk of reduced antiemetic response to ondansetron and tropisetron. Accordingly, the CPIC guideline gives a moderate recommendation to select an alternative antiemetic approach in CYP2D6 UMs.

Tamoxifen is an antiestrogenic agent that is used in treatment of breast cancer. It is bioactivated to 4-hydroxytamoxifen and endoxifen by CYP2D6 (Goetz, Sangkuhl, Guchelaar, Schwab, Province, Whirl-Carrillo et al., 2018). Compared to NMs, CYP2D6 PMs exhibit lower endoxifen concentrations and a higher risk of cancer recurrence when tamoxifen is used as an adjuvant in early breast cancer. The CPIC guideline for tamoxifen therefore recommends the use of alternative hormonal therapy for CYP2D6 poor metabolizers. For CYP2D6 IMs and NMs with an AS of 1, the recommendation is to consider alternative hormonal therapy, or if aromatase inhibitor use is contraindicated, to consider a higher but approved tamoxifen dose. For PMs, higher dose tamoxifen (40 mg/day) can be considered if there are contraindications to aromatase inhibitor therapy.

7. CYP3A subfamily

The CYP3A subfamily consists of four genes, namely *CYP3A4*, *CYP3A5*, *CYP3A7*, *CYP3A43* located on chromosome 7q22.1. CYP3A4 and CYP3A5 are abundantly expressed in the adult liver (Zhang et al., 2016). The expression of the other two isoforms is much lower, but CYP3A7 is abundantly expressed in fetal liver, contributing to about 50% of the total microsomal CYP protein content. CYP3A4 is important for the metabolism of about 50% of clinically used drugs including wide range of drugs and drug classes: benzodiazepines, calcium channel blockers, cyclosporine, macrolide antibiotics, opioids, several statins, and it also contributes to the metabolism of steroid hormones (S. F. Zhou, 2008). CYP3A4 is inhibited by a wide range of xenobiotics, such as erythromycin, grapefruit juice, ketoconazole, and HIV protease inhibitors. CYP3A4 is also highly inducible by PXR and CAR ligands. CYP3A5 is highly similar to CYP3A4, resulting in highly overlapping substrate selectivity.

7.1 CYP3A4

CYP3A4 activity is highly variable, but genetic variants in the *CYP3A4* gene have explained only a minor portion of this variability (Werk & Cascorbi, 2014). So far, the most relevant *CYP3A4* variant to explain CYP3A4 activity appears to be the intronic *CYP3A4**22 (rs35599367 C>T) variant, which occurs with a MAF of 5% in Europeans, and about 3% in admixed Americans, but is rare in many other populations (Y. Zhou et al., 2017). In 2011 it was shown to be associated with reduced CYP3A4 mRNA expression in liver samples, and with lower statin dose requirement for lipid control (D. Wang, Guo, Wrighton, Cooke, & Sadee, 2011). These findings have been subsequently replicated in several studies (Werk & Cascorbi, 2014). On the other hand, another widely studied variant, *CYP3A4**1B, appears to have no or very limited functional impact on CYP3A4 (García-Martín, Martínez, Pizarro, Garcia-Gamito, Gullsten, Raunio et al., 2002), in contrast to some initial reports which may have been influenced by its linkage disequilibrium with *CYP3A5**1. In addition to several non-synonymous exonic variants, two total loss-of-function variants have been described (*CYP3A4**20 and *26), both initially discovered based on single individuals with abnormal pharmacokinetics (Werk, Lefeldt, Bruckmueller, Hemmrich-Stanisak, Franke, Roos et al., 2014; Westlind-Johnsson, Hermann, Huennemeyer, Hauns, Lahu, Nassr et al., 2006). The frequency of these alleles is generally very low, but for example *CYP3A4**20 is present in the Spanish population with an allele frequency of 1.2% (Apellaniz-Ruiz, Inglada-Perez, Naranjo, Sanchez, Mancikova, Curras-Freixes et al., 2015).

The association of *CYP3A4**22 with reduced metabolic activity for several CYP3A4 substrates such as cyclosporine, erythromycin, sunitinib, tamoxifen, and quetiapine is well established (Werk & Cascorbi, 2014). Few studies have reported effects on pharmacodynamic endpoints for e.g., fluticasone in asthma and CYP3A4-metabolized statins in lipid control. However, no genotype-based dosing guidelines have been published for CYP3A4.

7.2 CYP3A5

CYP3A5 substrate selectivity overlaps with that of CYP3A4, and CYP3A5 contributes significantly to the CYP3A metabolic activity in individuals who express fully functional CYP3A5, i.e., in carriers of at least one *CYP3A5*1* allele (Kuehl, Zhang, Lin, Lamba, Assem, Schuetz et al., 2001). The most common and well-studied non-functional variant allele is the *CYP3A5*3* (Lamba, Hebert, Schuetz, Klein, & Altman, 2012). This allele is very common in admixed Americans, Europeans, East Asians, and South Asians, constituting the major allele with a frequency of 80%, 94%, 71%, and 67%, respectively (Y. Zhou et al., 2017). The allele frequency has been reported to correlate with the distance from the equator (Thompson, Kuttub-Boulos, Witonsky, Yang, Roe, & Di Rienzo, 2004), and it is considerably rarer in Africans (allele frequency 17%) than in other superpopulations. Other nonfunctional alleles are generally rare, but *CYP3A5*6* and **7* occur in African populations with allele frequencies of 15% and 10% (Y. Zhou et al., 2017).

The strongest evidence for clinical implications of *CYP3A5* variation is available for tacrolimus, which is one of the most widely used immunosuppressive medications in solid organ transplantation. Blood concentrations of tacrolimus have been associated with *CYP3A5* genotype in numerous studies, and CYP3A5 expressers have a higher tacrolimus clearance than non-expressers (Birdwell, Decker, Barbarino, Peterson, Stein, Sadee et al., 2015). Therapeutic drug monitoring is employed with tacrolimus therapy, and studies have shown that *CYP3A5* genotyping is helpful for achieving target trough whole blood concentrations when treatment is initiated. Less evidence is available on its effect on clinical outcomes. Furthermore, in liver transplant patients, both the genotype of the recipient and that of the donor liver can influence tacrolimus clearance, since CYP3A5 is expressed in both the intestine and the liver. However, the evidence is inconclusive at the moment regarding the relative significance of donor's and recipient's genotypes in liver transplants where the genotype is different between the two.

The evidence regarding *CYP3A5* genotype and statins metabolized by CYP3A enzymes is somewhat controversial, as some studies have shown poorer LDL-response to statin therapy in *CYP3A5* expressors than in non-expressors (Kivistö, Niemi, Schaeffeler, Pitkälä, Tilvis, Fromm et al., 2004), whereas other studies have implicated reduced response in *CYP3A5* non-expressors (Willrich, Hirata, Genvigir, Arazi, Rebecchi, Rodrigues et al., 2008). Furthermore, there is limited evidence that *CYP3A5**3 is associated with statin-induced myopathy. In one study, atorvastatin-associated myalgia cases with the *CYP3A5**3/*3 genotype had higher creatine kinase levels than those with the *CYP3A5**1/*3 genotype (Wilke, Moore, & Burmester, 2005). In addition, an association with the *CYP3A5* genotype and treatment-related toxicity has been reported for the anticancer agents sunitinib and vincristine, which are both metabolized by CYP3A enzymes (Egbelakin, Ferguson, MacGill, Lehmann, Topletz, Quinney et al., 2011; Garcia-Donas, Esteban, Leandro-Garcia, Castellano, del Alba, Climent et al., 2011). However, due to limited evidence, no other genotype-based dosing guidelines than those for tacrolimus have been published for *CYP3A5* to date.

8. Conclusion

Due to substantial research efforts, there has been a tremendous progress in understanding the genetic variability of drug-metabolizing CYP enzymes, their genotype-phenotype relationships, as well as their clinical significance. During the last decade, important advances have included

- the discovery and characterization of the clinical relevance of certain fairly common functionally relevant CYP alleles (particularly *CYP2C19**17 and *CYP3A4**22),
- recognition of the significance of CYP alleles common in individuals of non-European ancestry (e.g., certain decreased function *CYP2C9* alleles),
- understanding of the significance of rare loss-of-function CYP variants,

- realization that in all populations, the proportion that possess an “actionable pharmacogene” (i.e., a pharmacogenetic variant which is associated with actionable prescribing decision for a specific drug) is very high,
- detailed characterization of the clinical usefulness of pharmacogenetic approaches related to specific drugs (even prospective, randomized controlled clinical trials of pharmacogenetics versus conventional approaches have been conducted with warfarin),
- and perhaps most importantly the systematic production of drug specific evidence-based pharmacogenetic guidelines.

At the same time with increased understanding of the pharmacogenetics of CYP enzymes, there has been a rapid movement from traditional genotyping towards an era of large-scale exome and genome sequencing. This has already now increased the number of reported rare non-synonymous CYP variants to thousands (Fujikura, Ingelman-Sundberg, & Lauschke, 2015). Such variants of uncertain significance will pose a special challenge to pharmacogenetics, since they may have a very large impact on drug pharmacokinetics when they are present as a diplotype together with a common loss-of-function variant. Improvement of various computational approaches and large-scale functional assays will be needed to make significant progress in understanding the significance of such a multitude of rare variants, since there will be no possibility to investigate each of them clinically.

Despite all the scientific advances, the incorporation of pharmacogenetic information to clinical routine has been slow. So far, only few hospitals worldwide have adapted large-scale pre-emptive genotyping in clinical use. The reasons for the slow progress include organizational, ethical, legal and financial issues, in addition to medical issues. Sometimes, a main reason has been that alternative drugs with a perceived or known more reliable clinical effect have become preferred, in order to avoid pharmacogenetic complexities (Azmoon & Angiolillo, 2013).

In the future, the discovery and characterization of pharmacogenes and their variants will continue at a considerable rate, and work will be continued to compose and update pharmacogenetics consensus guidelines. In addition, research efforts will be needed to characterize the clinical utility and practicality of CYP genotyping in properly designed clinical trials. Moreover, incorporation of the pharmacogenetics guidelines to health care routines will require development of computational approaches, as well as electronic medical records and their interfaces, so that the use of pharmacogenetics information to support medical decisions is smooth and effective; a key challenge will be the provision of clear, unambiguous guidance to health care providers. At the same time, it should be kept in mind that in addition to genetic variability, CYP phenotypes are altered by multiple other individual factors, such as disease states and drug interactions.

Conflict of interest

The authors declare no conflict of interest.

Table 1. Drug metabolizing CYP enzymes, their chromosomal location, clinical significance of their genetic variability and examples of substrates. Substrates with an associated Clinical Pharmacogenetics Implementation (CPIC) guideline are given in bold.

Enzyme / gene	Chromosome	Significance of genetic variability	Examples of substrates
CYP1A2	15q24.1	+/-	Agomelatine, caffeine, clozapine, lidocaine, melatonin, tacrine, theophylline, tizanidine
CYP2A6	19q13.2	+	Cotinine, coumarin, letrozole, nicotine, tegafur
CYP2B6	19q13.2	+	Bupropion, efavirenz, cyclophosphamide
CYP2C8	10q23.33	+	Amodiaquine, cerivastatin, dasabuvir, imatinib, loperamide, montelukast, pioglitazone, paclitaxel, repaglinide, rosiglitazone
CYP2C9	10q23.33	++	Acenocoumarol, diclofenac, fluvastatin, glimepiride, glipizide, ibuprofen, losartan, phenytoin , S-warfarin
CYP2C19	10q23.33	++	Amitriptyline (and other tertiary amine tricyclic antidepressants) , clopidogrel, (es)citalopram, omeprazole, pantoprazole, sertraline , voriconazole
CYP2D6	22q13.2	++	Amitriptyline (and other tricyclic antidepressants) , aripiprazole, atomoxetine, codeine , dextromethorphan, fluvoxamine , metoprolol, mianserine, MDMA, ondansetron , paroxetine , propafenone, risperidone, tamoxifen , thioridazine, timolol, tropisetron , venlafaxine

CYP2E1	10q26.3	+/-	Ethanol, halothane, paracetamol
CYP2J2	1p32.1	+/-	Amiodarone, arachidonic acid, astemizole, cyclosporine
CYP3A4	7q22.1	+/-	Alfentanil, alprazolam, atorvastatin, budesonide, buspirone, cyclosporine, dexamethasone, erythromycin, felodipine, gefitinib, lovastatin, midazolam, nifedipine, quetiapine, saquinavir, sildenafil, simvastatin, tacrolimus, testosterone, triazolam, verapamil, vincristine
CYP3A5	7q22.1	++	Saquinavir, tacrolimus , verapamil, substrates overlap with CYP3A4

+/- Minor or no clinical significance

+ Moderate clinical significance

++ Major clinical significance

Table 2. Assignment of likely phenotypes based on examples of common diplotypes. Phenotype classification is based on consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC) (Caudle et al., 2017).

Phenotype	Examples of diplotypes			
	CYP2C9	CYP2C19	CYP2D6	CYP3A5
Ultrarapid or rapid metabolizer	-	*17/*17, *1/*17	(*1/*1)xN ^a , (*1/*2)xN, (*2/*2)xN	-
Normal metabolizer	*1/*1	*1/*1	*1/*1, *1/*2, *2/*2, *1/*9, *1/*41, *41/*41, *1/*5, *1/*4	*1/*1
Intermediate metabolizer	*1/*2, *1/*3	*1/*2, *1/*3, *2/*17 ^b	*4/*41, *5/*9, *4/*10	*1/*3
Poor metabolizer	*2/*2, *2/*3, *3/*3	*2/*2, *2/*3, *3/*3	*4/*4, (*4/*4)xN, *3/*4, *5/*5, *5/*6	*3/*3

^a xN represents the number of CYP2D6 gene copies.

^b provisional classification.

Table 3. Population frequencies of CYP2C9, CYP2C19, and CYP2D6 phenotypes in major populations.

	Poor metabolizer	Intermediate metabolizer	Normal metabolizer	Ultrarapid metabolizer ^a	Unknown / indeterminate ^b
CYP2C9					
Africans	1.8%	23%	75%	-	-
Europeans	4.0%	32%	64%	-	-
East Asians	0.1%	6.6%	93%	-	-
South Asians	4.5%	33%	62%	-	-
Americans	1.2%	20%	79%	-	-
CYP2C19					
Africans	4.8%	24%	17%	16%	38%
Europeans	2.5%	27%	39%	32%	0.0%
East Asians	14%	47%	37%	1.7%	0.2%
South Asians	12%	46%	23%	19%	0.0%
Americans	2.3%	26%	47%	24%	0.0%
CYP2D6					
Africans	1.9%	13%	72%	4.5%	9.2%
Europeans	6.1%	7.2%	75%	3.3%	8.5%
East Asians	0.9%	8.7%	86%	1.2%	3.7%
South Asians	1.3%	6.3%	89%	2.8%	0.4%
Americans	3.7%	4.5%	81%	4.8%	5.8%

^a For CYP2C19, rapid (*CYP2C19*1/*17*) and ultrarapid (*CYP2C19*17/*17*) metabolizers are combined.

^b For CYP2C9, phenotypes are based on genotyping for defining SNVs of the decreased function alleles *CYP2C9**2, *3, *5, *6, *8, and *11, and all alleles that are negative for these SNVs are defaulted to *CYP2C9**1. For CYP2C19 and CYP2D6, alleles whose functional status is indeterminate are included in the calculations.

Based on phenotype frequencies provided by PharmGKB and CPIC in Gene-specific Information Tables (<https://www.pharmgkb.org/page/cyp2c9RefMaterials>, <https://www.pharmgkb.org/page/cyp2c19RefMaterials>, <https://www.pharmgkb.org/page/cyp2d6RefMaterials>; accessed 6 February 2018)

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